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A non-staining chlorhexidine mouthwash? Probably not: a study *in vitro*

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Abstract *Background and aim:* Tooth staining is a common side effect of chlorhexidine mouth rinses and caused by the interaction of the di-cationic antiseptic with dietary chromogens. A product is now available, which claims an anti-discolouration system (ADS) with one clinical study in support. This study *in vitro* aims to determine whether two ADS rinses do or do not bind dietary chromogens. *Method and materials:* Optically clear acrylic specimens were cycled through human saliva (2 min), one of the three chlorhexidine rinses (two ADS and a positive control) (2 min) or water and then soaked in tea (60 min). After each cycle the optical density (OD) of specimens were read on a UV/visible spectrophotometer. The exit point was the cycle at which OD was >2.0. *Results:* All three rinses exceeded OD 2 at 11 cycles and there was no significant difference in staining for the ADS rinses compared with the positive control rinse. *Conclusion:* Based on extensive literature for the correlation of this test *in vitro* with chlorhexidine anti-plaque activity and propensity to stain *in vivo* these ADS rinses will have the same anti-plaque efficacy and potential to cause stain as established chlorhexidine rinse products.

Key words: chlorhexidine; extrinsic dental staining; mouth rinses; study *in vitro*; tea

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Introduction

It is now more than 30 years since chlorhexidine was shown to inhibit the formation of plaque and the development of gingivitis (1). Indeed, today chlorhexidine is still considered the 'Gold Standard' anti-plaque agent (for reviews see 2, 3). Early clinical studies identified local side effects of chlorhexidine formulations, which have tended to limit long-term use in preventive

dentistry (4). Perhaps best known is the development of extrinsic dental and tongue stain with all known efficacious chlorhexidine products, including mouth rinses (4), gels (5), sprays (5, 6), chewing gum (7, 8) and toothpaste (9, 10). The aetiology/mechanism of chlorhexidine staining can be debated, but evidence from numerous randomized controlled studies *in vivo* and *in vitro* demonstrate that an interaction of this di-cationic antiseptic with dietary chromogens on the tooth and mucosal surfaces is the major aetiological factor (for reviews see 3, 11, 12). Such a dietary mechanism also explains the dental and tongue staining seen with the oral use of other cationic antiseptics and polyvalent metal salts (13, 14).

The original and numerous studies have identified that the propensity of chlorhexidine to produce dietary staining *in vitro* correlated with both the efficacy against plaque and potential to cause stain *in vivo* (15–26). For example, a purported non-staining chlorhexidine mouth wash was shown to cause little staining in the laboratory model, referred to, but lacked clinical efficacy (19, 20). Interestingly, the manufacturers reformulated the mouth rinse in the UK but not in France. Subsequent studies revealed the UK formulation stained *in vitro* (21) and was efficacious against plaque and gingivitis (22). The French formulation remained non-staining *in vitro* and clinically ineffective (25, 26). Despite numerous attempts to produce non-staining chlorhexidine formulations two outcomes have always prevailed: inhibition of staining with loss of activity (27–30) or failure to prevent staining and maintenance of efficacy (31, 32). This has lead to the common statement about chlorhexidine products 'If it does not stain it does not work'. Recently, a claim for a reduced staining chlorhexidine mouth rinse has been made. The product contains an anti-discolouration system (ADS) based on ascorbic acid and sodium metabisulphite. An explanation, for the mechanism of action of the ADS, has not been given by the manufacturers, nor is it immediately apparent to us. This aside, a clinical study has been published reporting statistically significantly less stain with the 0.2% ADS chlorhexidine rinse compared with a 0.2% chlorhexidine positive control (33). The efficacy against plaque and gingivitis for the ADS rinse appeared unchanged, although one must assume a serious error in Table 2 of the paper (33) with mean and standard deviation values being identical for both plaque and gingivitis indices with both rinses. The study was crossover in design and therefore more suitable for a stain study of short duration than a parallel design, however there was no attempt to control diet and this could have confounded the results. Most studies on chlorhexidine staining have controlled at least beverage intake (14, 16, 17, 20, 25), indeed some models challenge the chlorhexidine with chromogenic beverages, for the 'forced stain model' (34–36). Such a model would more satisfactorily differentiate an ADS formulation from a positive control, if the ADS was effective. The present study used a well-accepted and proven method *in vitro*, which would demonstrate whether the ADS chlorhexidine rinses had reduced staining compared with a well-established UK chlorhexidine mouth rinse product.

Materials and method

The mouth rinses used were a 0.12 and 0.2% chlorhexidine products (Curasept; Curaden Healthcare srl, Saronno, Italy) (test) with ADS and a 0.2% chlorhexidine product (Corsodyl; GlaxoSmithKline, Oral Healthcare, Weybridge, UK) (positive control) and water (negative control).

The method is based on the original study of Addy *et al.* (15). Optically clear rectangular acrylic blocks (Perspex; Amari Plastics plc, Weybridge, UK) measuring 30 mm × 10 mm × 3 mm were prepared to fit the specimen chambers of a UV/visible double beam spectrophotometer. A baseline measurement for each block was taken at the maximum for tea of 295 nm. Groups of six blocks were allocated to each chlorhexidine solution and water control. Unstimulated human saliva was collected from the same individual at 9 AM and 2 PM each day. Each group of blocks were placed into saliva for 2 min, removed, washed in water and placed into the respective solution for 2 min. Specimens were then removed, washed and placed into a standard tea solution for 60 min. The standard tea solution was prepared by boiling 1 g of a single brand of tea leaves (Marks and Spencer Extra Strong, Marks and Spencer, London, UK) per 100 ml of water for 3 min, then decanted through gauze and cooled to room temperature. Finally, after removal from the tea solution, specimens were rinsed in water, allowed to bench dry and the optical density (OD) recorded on the spectrophotometer at 295 nm. The cycle was repeated until any one treatment solution resulted in an average OD for the group of >2.0. For the water control the same cycling procedure was followed but OD readings were only taken at the cycle at which one of the chlorhexidine groups reached OD >2.

Statistical methods

Based on previous data that there would be little staining in the water control group, it was decided *a priori* to perform analysis of variance across the chlorhexidine mouth rinse products at the exit cycle. If significant, paired comparisons would be performed using *t*-tests. Differences between the test products and water were determined using the Mann–Whitney test

together with the calculation of the point estimate and the construction of 95% confidence intervals.

Results

The mean and standard deviation values of the OD of groups of specimens at each cycle of treatment with each of the chlorhexidine rinses are shown in Table 1 together with the OD for the water control group at cycle 11. All chlorhexidine rinses showed a progressive increase in OD indicative of increased staining. All groups exceeded the OD exit point of >2 at cycle 11. As expected, comparatively little staining was seen with the water regimen. In mean terms, least staining was seen with the positive control and most with the 0.2% ADS chlorhexidine rinse, however statistically no significant differences in staining between the three rinses was apparent ($P > 0.05$) and further *post hoc* paired comparisons were not conducted. All chlorhexidine rinses stained significantly greater than water [$P = 0.0022$, point estimate 1.0 (maximum value), 95% confidence interval 0.69–1.0].

Discussion

As stated, over nearly three decades this methodology *in vitro* for chlorhexidine staining has correlated with not only staining potential *in vivo* but clinical anti-plaque efficacy (15–26). Various aspects of the model which has been performed over the time period of the method cannot be debated, except to confirm that the staining *in vitro* for the acrylic substrate correlates with human enamel *in vitro* (13, 14). This is not surprising given the early reports of similar staining on dentures as seen on teeth (37). The use of water as a negative control merely serves to confirm the conduct of the study indicating that the stain was because of the chlorhexidine and not the aqueous vehicle.

The data from this study completely refute the findings of the stain aspect of the clinical investigation (33) and confirm that both products with the ADS would cause staining *in vivo* and would have the same efficacy as conventional chlorhexidine mouth rinse products. There was little difference between the 0.12 and 0.2% ADS products, which is not surprising as there was well in excess of the dose of chlorhexidine to saturate, probably as a stable monolayer (38, 39), the entire surface of the specimens. Evidence from laboratory and clinical studies indicate that it is the chlorhexidine adsorbed to the tooth surface, which accounts for the plaque inhibition (40–42).

In conclusion, the present study *in vitro* correlates with the plaque and gingivitis data of the cited study (33) namely that

Table 1. **The optical density of specimens after each stain cycle for the chlorhexidine mouth rinses**

Product	Sample	Baseline	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Cycle 9	Cycle 10	Cycle 11
Test ADS	Mean	0.033	0.123	0.214	0.399	0.686	0.838	1.282	1.342	1.432	1.478	1.664	2.504
(0.12% CHX)	(SD)	(0.035)	(0.050)	(0.057)	(0.053)	(0.038)	(0.126)	(0.204)	(0.192)	(0.129)	(0.131)	(0.232)	(0.554)
Test ADS	Mean	0.023	0.140	0.222	0.346	0.679	0.904	1.400	1.456	1.554	1.888	1.988	2.798
(0.20% CHX)	(SD)	(0.016)	(0.016)	(0.022)	(0.041)	(0.149)	(0.100)	(0.097)	(0.166)	(0.160)	(0.360)	(0.335)	(0.396)
Positive control	Mean	0.033	0.143	0.269	0.449	0.844	1.301	1.445	1.583	1.723	1.793	1.880	2.344
(0.2% CHX)	(SD)	(0.031)	(0.027)	(0.050)	(0.096)	(0.159)	(0.237)	(0.177)	(0.108)	(0.094)	(0.063)	(0.059)	(0.364)
Water	Mean												0.193
	(SD)												(0.082)

the formulation with the ADS should be as efficacious as well established chlorhexidine mouth rinse products. Unfortunately, these data contradict the conclusion that the ADS products have the potential to reduce dental staining. Indeed, these ADS products would be expected to have identical staining potential to all other efficacious chlorhexidine rinses. More clinical studies are necessary to confirm the conclusion, which use more controlled protocols particularly in respect of dietary chromogen intake.

References

1 Loe H, Schiott CR. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. *J Periodontal Res* 1970; **5:** 79–83.

2 Jones CG. Chlorhexidine: is it still the gold standard? In: Addy M, Moran JM, ed. *Toothpaste, Mouthrinse and Other Topical Remedies in Periodontics. Periodontol 2000* 1997; **15:** 55–62.

3 Addy M. The use of antiseptics in periodontal therapy. In: Lindhe J, Karring T, Lang K, ed. *Clinical Periodontology and Implant Dentistry*, Chapter 22. Blackwell Munksgaard: Copenhagen. 2003: 464–493.

4 Flotra L, Gjermo P, Rolla G, Waerhaug J. Side effects of chlorhexidine mouthwashes. *Scand J Dent Res* 1971; **79:** 119–125.

5 Francis JR, Hunter B, Addy M. A comparison of three delivery methods of chlorhexidine in handicapped children. I. Effects on plaque, gingivitis and toothstaining. *J Periodontol* 1987; **58:** 451–454.

6 Kalaga A, Addy M, Hunter B. The use of 0.2% chlorhexidine as an adjunct to oral health in physically and mentally handicapped adults. *J Periodontol* 1989; **60:** 381–385.

7 Ainamo J, Etemadzadeh H. Prevention of plaque growth with chewing gum containing chlorhexidine acetate. *J Clin Periodontol* 1987; **14:** 524–527.

8 Smith A, Moran J, Dangler LV, Leight RS, Addy M. The efficacy of an antigingivitis chewing gum. *J Clin Periodontol* 1996; **23:** 19–23.

9 Yates R, Jenkins S, Newcombe RG, Wade WG, Moran J, Addy M. A 6-month home usage trial of a 1% chlorhexidine toothpaste. 1. Effects on plaque, gingivitis, calculus and tooth staining. *J Clin Periodontol* 1993; **20:** 130–138.

10 Sanz M, Vallcorba N, Fabregues S, Muller I, Herkstroter F. The effect of a dentifrice containing chlorhexidine and zinc on plaque, gingivitis, calculus and tooth staining. *J Clin Periodontol* 1994; **21:** 431–437.

11 Watts A, Addy M. Tooth discolouration and staining: a review of the literature. *Brit Dent J* 2001; **190:** 309–316.

12 Addy M, Moran J. Mechanisms of stain formation on teeth, in particular associated with metal ions and antiseptics. *Advances Dent Res* 1996; **9:** 450–456.

13 Addy M, Moran J, Griffiths A, Wills-Wood NJ. Extrinsic tooth discolouration by metals and chlorhexidine. I: surface protein denaturation or dietary precipitation? *Brit Dent J* 1985; **159:** 331–334.

14 Addy M, Moran J M. Extrinsic tooth discolouration by metals and chlorhexidine. 2. Clinical staining produced by chlorhexidine iron and tea. *Brit Dent J* 1985; **159:** 331–334.

15 Addy M, Prayitno S, Taylor L, Cadogan S. An *in vitro* study of the role of dietary factors in the aetiology of tooth staining associated with the use of chlorhexidine. *J Periodontal Res* 1979; **14:** 403–410.

16 Prayitno S, Taylor L, Cadogan S, Addy M. An *in vivo* study of dietary factors in the aetiology of tooth staining associated with the use of chlorhexidine. *J Periodontal Res* 1979; **14:** 411–417.

17 Roberts WR, Addy M. Comparison of the bisbiguanide antiseptics alexidine and chlorhexidine. I: effect on plaque accumulation and salivary bacteria. *J Clin Periodontol* 1981; **8:** 213–219.

18 Addy M, Roberts WR. Comparison of the bisguanide antiseptics alexidine and chlorhexidine II. Clinical and *in vitro* staining properties. *J Clin Periodontol* 1981; **8:** 220–230.

19 Addy M, Wade WG, Jenkins S, Goodfield S. Comparison of two commercially available chlorhexidine mouthrinses. I. Staining and anti-microbial effects. *Clin Prev Dent* 1989; **11:** 10–14.

20 Jenkins S, Addy M, Newcombe RG. Comparison of two commercially available chlorhexidine mouthrinses. II. Effects on plaque reformation, gingivitis and toothstaining. *Clin Prev Dent* 1989; **11:** 12–16.

21 Addy M, Wade W, Goodfield S. Staining and antimicrobial properties in vitro of some chlorhexidine formulations. *Clin Prev Dent* 1990; **12:** 13–17.

22 Addy M, Moran JM, Newcombe R. A comparison of 0.12% and 0.1% chlorhexidine mouthrinses in the development of plaque and gingivitis. *Clin Prev Dent* 1991; **13:** 26–29.

23 Mendieta C, Vallcorba N, Binney A, Addy M. Comparison of two chlorhexidine mouthwashes on plaque regrowth in vivo and dietary staining in vitro. *J Clin Periodontol* 1994; **21:** 296–300.

24 Smith R, Moran J, Addy M, Doherty F, Newcombe RG. Comparative staining in vitro and plaque inhibiting properties in vivo of 0.12% and 0.2% chlorhexidine mouthrinses. *J Clin Periodontol* 1995; **22:** 613–617.

25 Addy M, Wade WG. An approach to efficacy screening of mouthrinses: studies on a group of French products (I). Staining and antimicrobial properties in vitro. *J Clin Periodontol* 1995; **22:** 718–722.

26 Renton-Harper PR, Milsom S, Wade WG, Addy M, Moran J, Newcombe RG. An approach to efficacy screening of mouthrinses: studies on a group of French products (II). Inhibition of salivary bacteria and plaque in vivo. *J Clin Periodontol* 1995; **22:** 723–727.

27 Wade W, Slayne MA, Addy M. The antibacterial and antistaining properties of a novel antiadherent agent M239144 alone and in combination with chlorhexidine. *J Clin Periodontol* 1994; **21:** 438–440.

28 Moran J, Addy M, Newcombe R, Warren P. The comparative effects on plaque regrowth of phenolic, chlorhexidine and antiadhesive mouthrinses. *J Clin Periodontol* 1995; **22:** 929–934.

29 Addy M, Moran M, Newcombe R, Warren P. The comparative tea staining potential of phenolic, chlorhexidine and antiadhesive mouthrinses. *J Clin Periodontol* 1995; **22:** 919–924.

30 Claydon N, Addy M, Ridge D, Jackson R. An evaluation of an antiadhesive copolymer agent on plaque inhibition by chlorhexidine. *J Clin Periodontol* 1996; **23:** 952–954.

31 Claydon N, Addy M, Jackson R, Smith S, Newcombe RG. Studies on the effect of polyvinyl pyrrolidone on the activity of chlorhexidine mouthrinses: plaque and stain. *J Clin Periodontol* 2001; **28:** 558–564.

32 Claydon N, Manning CM, Darby-Dowman A, Ridge D, Smith S, Addy M. The effect of polyvinyl pyrrolidone on the clinical activity of 0.09% and 0.2% chlorhexidine mouthrinses. *J Clin Periodontol* 2001; **28:** 1037–1044.

33 Bernadi F, Pinelli MR, Carloni S, Gatto MR, Montebugnoli L. Chlorhexidine with an anti discoloration system. A comparative study. *Int J Dent Hygiene* 2004; **2:** 122–126.

34 Moran J, Claydon NCA, Newcombe RG, Addy M. Clinical study to determine the effectiveness of a whitening toothpaste at reducing stain using a forced stain model. *J Clin Periodontol* 2004; **31:** 1088–91.

35 Claydon NCA, Moran J, Bosma M, Shirodna S, Newcombe RG, Addy M. A clinical study to compare the effectiveness of a test whitening toothpaste with a commercial whitening toothpaste at inhibiting dental stain. *J Clin Periodontol* (in press).

36 Pontefract H, Courtney M, Smith S, Newcombe RG, Addy M. The development of methods to enhance extrinsic tooth discolouration for comparison of toothpastes. 2. Two product clinical study. *J Clin Periodontol* 2004; **31:** 7–11.

37 Olsen I. Relapse tendency and removal of acquired discolourations in long term denture disinfection with chlorhexidine. *Acta Odont Scand* 1975; **33:** 111–114.

38 Emilson CG, Eriksen TH, Heyden G, Magnusson BC. Uptake of chlorhexidine to hydroxapatite. *J Periodontal Res* 1973; **8 (Suppl. 12):** 17–21.

39 Addy M, Roberts WR. The use of polymethylmethacylate to compare the adsorption and staining reactions of some cationic antiseptics. *J Periodontol* 1981; **52:** 380–385.

40 Kalaga A, Addy M, Hunter B. Comparison of chlorhexidine delivery by mouthwash and spray on plaque accumulation. *J Periodontol* 1989; **60:** 127–130.

41 Jenkins S, Addy M, Wade W. The mechanism of action of chlorhexidine: a study of plaque growth on enamel inserts in vivo. *J Clin Periodontol* 1988; **15:** 415–424.

42 Davies RM, Jensen SB, Schiott CR, Loe H. The effect of topical application of chlorhexidine on the bacterial colonization of the teeth and gingiva. *J Periodontal Res* 1970; **5:** 96–101.